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Divide and Conquer: Division of Labor by B-1 B Cells

Capsular polysaccharides enhance bacterial virulence. In this issue of *Immunity*, Haas et al. (2005) demonstrate a division of labor between the B-1 B cell subsets: natural antibodies from B-1a cells limit infection by *Streptococcus pneumoniae*, whereas B-1b cells generate the anticapsule-induced response that prevents fatal infection.

Pathogens employ a variety of strategies to overcome host immune surveillance. Clinically important bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* express polysaccharide (PS) capsules. These PS capsules are T cell-independent type-2 (TI-2) antigens, have limited intrinsic immunogenicity, and can also mask immunogenic surface molecules, thus enabling persistence of these pathogens in the host. A critical factor in preventing systemic infection is the ability of B cells to rapidly generate protective antibodies against capsular PS. Pneumococcal PS can elicit protective immunity, but vaccine development is complicated by the existence of more than 90 serotypes and their geographical distribution. The PS vaccine against *S. pneumoniae* contains 23 prevalent PS serotypes including the PS3 used in Haas et al. (2005). This PS vaccine generates effective antibody responses in adults but not in children. The lack of vaccine anti-PS response in the children under the age of 2 years can be overcome by the covalent conjugation of PS (from a subset of serotypes) to a protein carrier to elicit T cell-dependent responses (Peltola et al., 2004). Despite this progress, it is still important to understand the mechanisms underlying children's hypersusceptibilty to pneumococcal infection. The work from Haas et al. (2005) identified the B cell subsets and humoral immune mechanisms responsible for anti-PS antibody responses using a mouse model.

Murine mature B cells are heterogeneous and belong to four subsets: follicular (FO), marginal zone (MZ), B-1a ($CD5^+$), and B-1b ($CD5^-$). FO B cells recirculate in blood and among the lymphoid follicles and mount antibody responses against T cell-dependent protein antigens (reviewed in [Martin and Kearney, 2001]). Both MZ B cells, localized proximal to the marginal sinus of the spleen, and B-1 cells, most abundant in peritoneal and pleural cavities, generate T cell-independent responses. B-1a cells are the major source of natural antibodies that occur spontaneously in naive, "antigen-free" mice, and natural antibodies are important in controlling pathogenic viruses and bacteria including *S. pneumoniae* (reviewed in [Baumgarth et al., 2005]). However, a specific role for B-1b cells, generating immunity to the relapsing fever bacterium *Borrelia hermsii,* was only recently found (Alugupalli et al., 2004).

In this issue of *Immunity*, Haas et al. (2005) utilize the well-established murine model of *S. pneumoniae* infection to compare mice sufficient, deficient, or overexpressing CD19: conveniently, they establish that $CD19^{-/-}$ mice have an intact B-1b population and a severe deficiency in B-1a and MZ cells, yet mice overexpressing human CD19 (hCD19) have B-1a cells but have severely reduced B-1b cells. Surprisingly, they find CD19 is not required for generating protective IgM or IgG3 responses against the PS of *S. pneumoniae*. This observation, and analyses of B cell populations in the $CD19^{-/-}$ or hCD19 mice, indicate that B-1b cells make the protective anti-PS response. By adoptive transfer of purified B cell subsets into immunodeficient ($rag^{-/-}$) mice, Haas et al. (2005) demonstrate that B-1b cells but not B-1a cells generate the IgM and IgG3 response essential and sufficient to prevent lethal infection by *S. pneumoniae*. However, B-1a cells have an important role: naive hCD19 mice survive when challenged with a sublethal dose of *S. pneumoniae*, *S. pneumoniae* natural antibodies are intact in hCD19 mice and transferred serum from wild-type mice confers protection of $CD19^{-/-}$ mice. Importantly, transferred B-1a cells spontaneously produce low levels of PS-reactive IgM (but not IgG3) antibody. Therefore, to combat *S. pneumoniae,* B-1 subsets collaborate: B-1a cells secrete natural antibody that protects against infection or lowers bacterial burden if infection is established, whereas B-1b cells secrete the induced antibody needed to clear bacteria and permit survival.

The molecular rules for this division of labor are as yet undefined. Although CD5 expression is the phenotypic criterion used to distinguish B-1a from B-1b subsets, several biological distinctions exist. Defects in BCR signaling more profoundly affect B-1a cell development (Hardy and Hayakawa, 2001). The Haas et al. (2005) paper builds on prior evidence that CD19 expression is essential for the development of B-1a but not B-1b cells. *xid* mice (defective in BCR signaling due to a mutation in Bruton's tyrosine kinase) have defects in both B-1a and B-1b cells, although introduction of IL-9 as a transgene rescues B-1b but not B-1a development (Knoops et al., 2004). The Ig repertoires of B-1a versus B-1b are also distinct. Interestingly, single-cell unbiased samples reveal that B-1a cells have fewer N region additions at VH-D and D-JH Ig gene junctions than B-1b cells (Kantor et al., 1997). This distinction between N region frequencies is consistent with findings that bone marrow progenitors express Tdt, the enzyme responsible for N regions, and have progenitor activity for B-1b but not B-1a cells. By adoptive transfer, we found bone marrow sufficient for the generation of B-1b cells capable of eradicating *B. hermsii* (Alugupalli et al., 2004). Therefore, different Ig repertoires, requirements for signals to supplement BCR signaling or as yet unappreciated intrinsic differences could each contribute to the unique competency of B-1b cells to mount the protective anti-PS response.

The generation of B cell memory has been regarded as exclusively T cell dependent. We recently found that B-1b cells generate long-lasting IgM memory in T cell-deficient mice infected with *B. hermsii*. The present pa-

per demonstrates that B-1b cells generate long-lasting T cell-independent immunity to *S. pneumoniae*. It will be interesting to determine mechanisms that promote T-independent memory against these bacteria. Presumably, pathogens engage many more components of the immune system than synthetic pure TI-2 antigens such as haptenated ficoll (the widely used model TI-2 antigen). It has been appreciated for some time that TI *S. pneumoniae*-specific plasmablast generation relies on Ag-capture and antigen-presenting cell (APC)-derived signals such as BLyS for both MZ B and B-1a responses (Balazs et al., 2002). These APCs must interact with bacteria to activate their accessory function for B cells, suggesting a role for innate signals in priming the TI response (reviewed in [Vos et al., 2000]). Do such signals also contribute to B-1b cell memory?

The generation of long-lasting immunity to *S. pneumoniae* in the mouse model suggests new avenues for vaccine research. *S. pneumoniae* remains a major worldwide pathogen, with over 1 million deaths per year, mainly in children. Children and the elderly also respond poorly to the PS vaccine. The reasons for this are not clear. Children and splenectomized-individuals are more susceptible to recurrent infections by encapsulated bacteria, perhaps due to the lack of $CD21^+$ MZ B cell development in the spleens of children under 2 years of age, as CD21 is vital for MZ B TI-2 responses (Zandvoort et al., 2001). The majority of B cells in young children express CD5 [reviewed in (Baumgarth et al., 2005)]. While it is not clear that all distinctions between B cell subsets defined in the mouse hold true for humans, it is tempting to consider that children might lack sufficient protection from a B-1b-type cell. Natural antibodies and IgM memory B cells are implicated in protection against pneumococci in humans (Carsetti et al., 2005), suggesting parallels to the groundwork laid by Haas et al. (2005) in this issue.

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